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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

CASSELS, et al.

Serial No.: 09/942,974

Filed: 31 August 2001

For:

MASS SPECTROMETRY OF COLONIZATION
FACTORS

Art Unit: 1651

Examiner: GITOMER, Ralph J.

Atty. Docket: 034047.0342
(WRAIR 97-30B)

**DECLARATION OF FREDERICK J. CASSELS, Ph.D.
UNDER 37 C.F.R. 1.132**

1. I, Frederick J. Cassels, am a citizen of the United States of America and I reside at 10435 E.B. White Court, Laurel, MD 20723.
2. I am an inventor of the above-referenced patent application, U.S. Patent Application Serial No. 09/942,974 ('974 application), filed 31 August 2001.
3. The '974 application is a continuation-in-part of U.S. Patent Application Serial No. 09/580,385 ('385 application), filed 26 May 2000, which is a continuation-in-part of U.S. Patent Application Serial No. 09/070,802 ('802 application), filed 1 May 1998, which claims the benefit of U.S. Provisional Patent Application Serial No. 60/045,511 ('511 application), filed 2 May 1997.
4. I am also an inventor of the applications listed in #3 above.
5. The '511 application does not disclose the subject matter claimed in the present application, but the '802 and '385 applications as originally filed do disclose the subject matter as claimed in the present application.
6. Attached herewith are two mass spectrographs. The first is of a colonization factor first dissolved in acetic acid and then hexafluoropropanol added second. The second is of a colonization factor first dissolved in hexafluoropropanol and then acetic acid added second.
7. The first mass spectrograph shows that dissolving the colonization factor in acetic acid first does not provide an observable signal of the 15055 and 15877 protein peaks.
8. The second mass spectrograph shows that dissolving the colonization factor in hexafluoropropanol first provides observable signals of the 15055 and 15877 protein peaks.
9. The 15055 and 15877 protein peaks are necessary for correctly identifying colonization factors, such as CS6, via mass spectrometry.

10. The abstract for ASM General Meeting entitled "Absolute Molecular Weight Determination of *E. coli* Fimbrial Major Subunits" (1993) by Cassels et al. is attached herewith. The abstract does not teach or disclose that colonization factors should first be dissolved in hexafluoropropanol and to which acetic acid is then added.

11. In my opinion, the abstract does not teach the criticality of first, solubilizing the colonization factor by dissolving the colonization factor in 1,1,1,3,3,3-hexafluoro-2-propanol and then second, adding a solution of volatile acid to the solubilized colonization factor to obtain a product in order to identify colonization factors via mass spectrometry.

12. Therefore, in my opinion, the abstract does not teach or suggest a method for identifying at least one bacterial colonization factor via mass spectrometry wherein the colonization factor is first, solubilized in 1,1,1,3,3,3-hexafluoro-2-propanol and then second, a solution of volatile acid is added to the solubilized colonization factor.

13. Clearly, in my opinion, the abstract does not teach or suggest a method for identifying at least one bacterial colonization factor of enterotoxigenic *E. coli* which comprises the following steps in the following order:

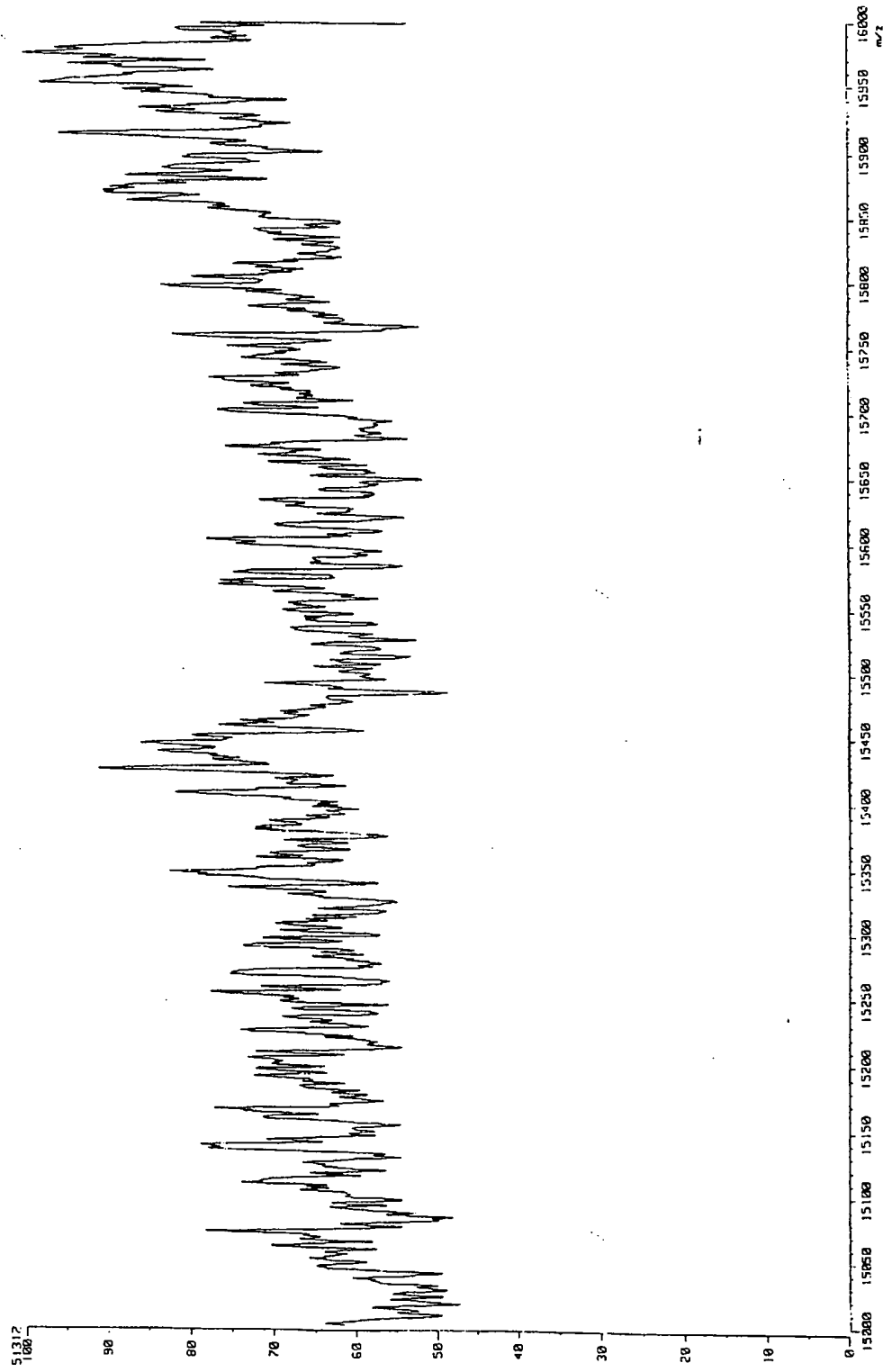
- 1) obtaining the colonization factor;
- 2) solubilizing the colonization factor by dissolving the colonization factor in 1,1,1,3,3,3-hexafluoro-2-propanol;
- 3) adding a solution of volatile acid to the solubilized colonization factor of step 2 to obtain a product;
- 4) subjecting the product of step 3 to mass spectrometry to determine the mass of the colonization factor; and
- 5) comparing the mass determined in step 4 with the mass of at least one known colonization factor.

14. I hereby state that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issuing thereon.

EXECUTED at Silver Spring this 11th day of May 2004,

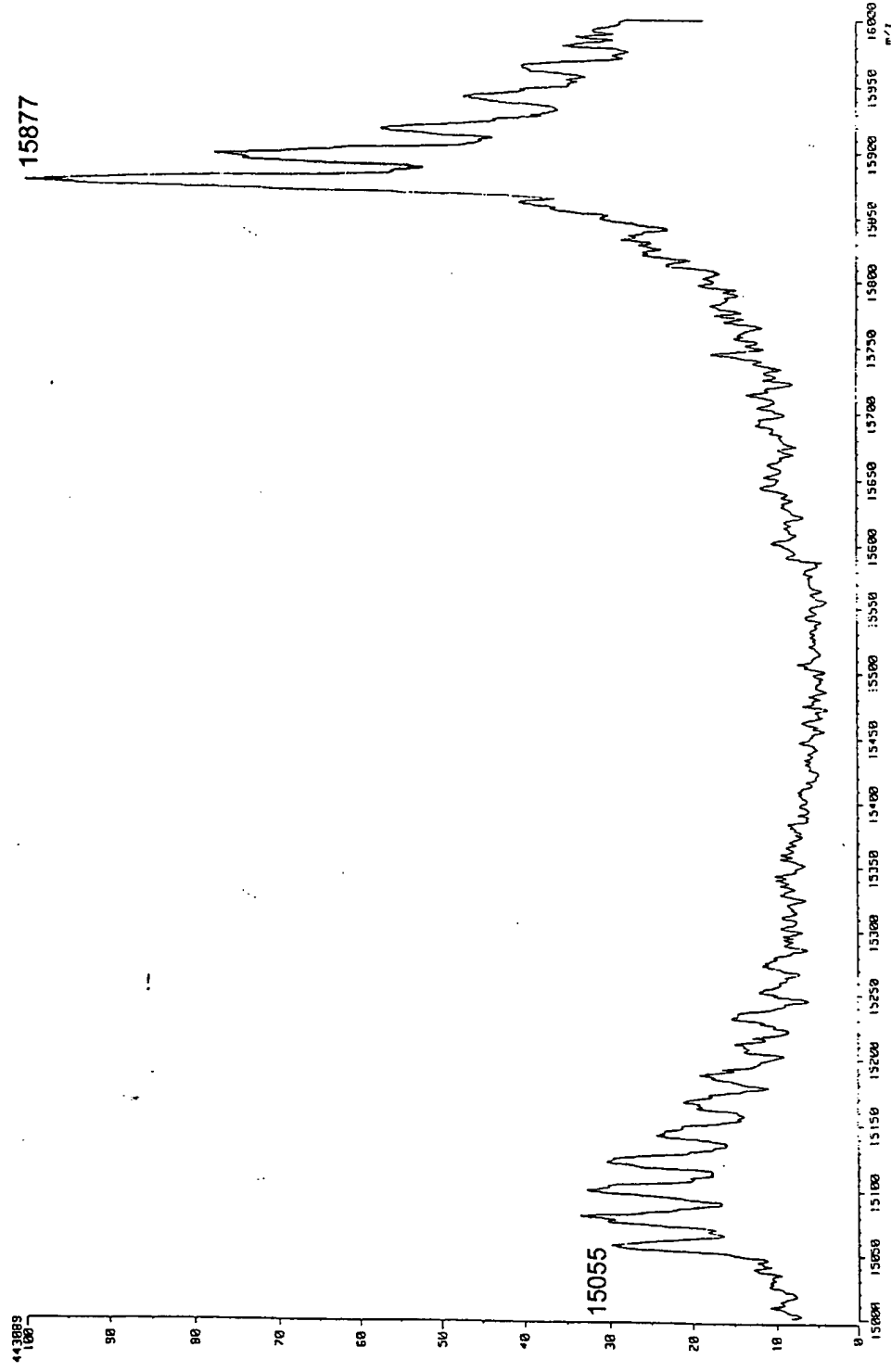
By Frederick J. Cassels
Frederick J. Cassels

CS6 First Dissolved in 5% Acetic Acid
Followed by an Equal Volume of Hexafluoropropanol



Note: There is no sign of the 15055 and 15877 protein peaks

CS6 First Dissolved in Hexafluoropropanol
Followed by an Equal Volume of 5% Acetic Acid



- Note: 1. The protein peaks are clearly seen along with their sodium adducts
2. The background (e.g. m/z 15500) is roughly constant with previous example